Correlation of Environmental Carbaryl Measurements with Serum and Urinary 1-Naphthol Measurements in a Farmer Applicator and His Family

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In exposure or risk assessments, both environmental and biological measurements are often used. Environmental measurements are an excellent means for evaluating regulatory compliance, but the models used to estimate body burden from these measurements are complex. Unless all possible routes of exposure (i.e., inhalation, dermal absorption, ingestion) are evaluated, exposure to a toxicant can be underestimated. To circumvent this problem, measurements of the internal dose of a toxicant in blood, serum, urine, or tissues can be used singularly or in combination with environmental data for exposure assessment. In three separate laboratories, carbaryl or its primary metabolite, 1-naphthol, was measured in personal air, dermal samples, blood serum, and urine from farmer applicators and their families. The usefulness of both environmental and biological data has been demonstrated. For the farmer applicator, the environmental levels of carbaryl would have been sufficient to determine that an exposure had occurred. However, biological measurements were necessary to determine the absorbed dose of each member of the applicator's family. In addition, a correlation between serum and urinary 1-naphthol measurements has been shown; therefore, either matrix can be used to accurately evaluate occupational carbaryl exposure. *Key words:* air, carbaryl, farmer, 1-naphthol, serum, urine. *Environ Health Perspect* 105:510–513 (1997)

Carbaryl is one of the most widely used industrial insecticides (*1*). In 1988, about 25 million pounds of carbaryl was applied to crops on farms in the United States to eliminate chewing and sucking insects (*1*). Carbaryl does not generally linger in the environment. It is readily absorbed into the soil, where it quickly breaks down, and does not leach into groundwater. For these reasons, residual levels of carbaryl in the environment are not believed to be hazardous to the general population.

Agricultural workers, however, are often exposed to much greater levels of carbaryl than the general population. Because exposure symptoms associated with cholinesterase inhibition usually appear long before toxic quantities of carbaryl are absorbed, acute poisonings involving carbaryl are rare. Although toxic quantities are rarely absorbed in occupational settings, improper use of safety equipment (i.e., gloves, respirators, protective clothing) may lead to high-level exposures to carbaryl.

In humans, carbaryl does not accumulate in tissues or persist in blood. It is quickly metabolized into a nontoxic compound, 1-naphthol, which is excreted in urine as the glucuronide or sulfate ester (*2*). The body burden of 1-naphthol as measured in urine or serum is the most common indicator of exposure to carbaryl.

Carbaryl exposure may also be evaluated indirectly by measuring carbaryl in personal respiratory air, dermal patches, and dermal wipes. Although these environmental measurements are not necessarily related to the amount of carbaryl absorbed by the body, they provide useful information about exposure routes and absorption potential. However, if data are obtained that show a clear correlation between environmental and biological measurements, that correlation could be used to evaluate the mechanisms of carbaryl entry into and distribution within the body.

Environmental measurements of carbaryl and biological measurements of 1-naphthol in selected agricultural farmer applicators and their families are reported. These data, when viewed collectively, provide useful information for occupational carbaryl exposure assessment.

Materials and Methods

Sample collection. All samples were collected from six farmer applicators and their families, who voluntarily, with informed consent, participated in the pilot Agricultural Health Study (*3*) conducted by the National Cancer Institute (NCI), the U.S. Environmental Protection Agency (EPA), and the National Institute of Environmental Health Science (NIEHS). In this study, biological (i.e., blood and urine) and environmental (i.e., air, house dust, food) residues of a variety of pesticides and/or their metabolites were measured and detailed questionnaire data obtained. The collective data obtained in this study will assist the cosponsoring agencies evaluate the role of agricultural exposures, as well as dietary and lifestyle factors, in the development of cancers and of neurologic and other chronic diseases (*3*). The final interpretation of the collective study data will be reported elsewhere. Only the data from biological and personal environmental samples involving carbaryl exposures will be discussed here.

During the application season, up to four biological samples were collected from each participant and two personal air, dermal, and handwipe samples were collected from the farmer applicator. One sample was collected on the day prior to pesticide application and another on the day of pesticide application. Biological samples were also collected on the two days following pesticide application. Additional environmental samples included one handwipe sample from each family member and one household indoor air sample taken on the day of application. Personal air samples were not obtained from the family members because previous studies (*4,5*) suggest that concentrations of toxicants in their personal air are similar to indoor air concentrations.

Personal air, dermal patch, and handwipe analysis. Personal air and dermal samples were collected only from the farmer applicator. In accordance with established sampling procedures, the applicator wore a portable air sampler and an α-cellulose dermal patch during the entire sampling period (*4*). Handwipe samples were collected from the applicator and family members with cotton swabs and isopropanol (*4*). Following a previously published solvent extraction

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procedure (5), carbaryl was isolated from sample matrices and analyzed by gas chromatography with mass spectrometry. The limits of detection (LODs) for the personal air, dermal patch, and handwipe analyses were 0.7 ng/m^3, 14.3 ng/sample, and 1.74 ng/sample, respectively. The coefficients of variation (CVs) ranged from 5 to 15%.

Urine analysis. Urine samples (10 ml) were prepared according to a previously published method that involved solvent extraction and chemical derivatization (6). The derived extracts were analyzed by gas chromatography coupled with isotope-dilution, low-resolution tandem mass spectrometry (7). Two ions were monitored for both the native 1-naphthol and the ^{13}C-labeled analogue. One ion was used for 1-naphthol quantification and the other for analyte confirmation. The calculated concentrations of unknowns were normalized on the creatinine content in each urine specimen. The LOD [calculated as three times the standard deviation at zero concentration($3s_0$)] of the method was 1.2 µg/l (ppb) with an average CV of 7.5% on repeat measurements of unknown samples at concentrations spanning the entire linear range.

Serum analysis. Serum samples (8 g) were prepared according to an established method (J.R. Barr, unpublished data) involving protein denaturation and solid phase extraction (SPE). 1-Naphthol concentrations in the serum extracts were analyzed by gas chromatography and isotope-dilution, high-resolution mass spectrometry (J.R. Barr, unpublished data). The LOD ($3s_0$) of the method was 19 ng/l (ppt) with an average CV of 19% at 100 ng/l.

Data analysis. All data were evaluated statistically with SAS statistical software (SAS Institute, Cary, NC). Most of the data points available for correlation analyses were very near the LOD. To avoid using a very skewed distribution of data points in statistical analyses, only quantitative data above the limit of quantitation (LOQ; $10s_0$) (8) of the biological methods were used in correlation evaluations. The elimination of the data between the LOD and LOQ did not critically affect the correlation analysis or its significance. Pearson correlations were considered significant if $p<0.05$.

Results and Discussion

Of the six farmer applicators studied, only one was actively applying carbaryl on his crops at the time of monitoring. The carbaryl and 1-naphthol concentrations measured in environmental and biological samples associated with this applicator are shown in Table 1. Only biological data (not shown) were obtained from those farm applicators and families from farms on which carbaryl was not applied during the study. The concentrations of 1-naphthol in the urine samples from these individuals exhibited no elimination pattern and were below the 95th percentile of the reference range, suggesting only background exposure.

Table 1. Concentrations of carbaryl in the environmental samples and 1-naphthol and carbaryl in biological samples from a carbaryl applicator

Sample	Preapplication day	Application day	Post application day 1	Post application day 2
Personal air (µg/m^3 carbaryl)	0.0080[a] 0.016[b]	640	NS	NS
Dermal patch (µg/cm^2 carbaryl)	0.010[a] 0.0014[b]	11	NS	NS
Handwipe (µg carbaryl)	20.0[a] 9.0[b]	20,100	NS	NS
Urine[c] (µg/g creatinine 1-naphthol)	270 (860)	140 (500)[a] 9,300 (22,000)[b]	7,100 (12,000)	1,500 (2,600)
Serum (µg/l 1-naphthol)	0.260	510	1.9	0.56
Serum (µg/l carbaryl)	ND	0.12	ND	ND

Abbreviations: NS, no sample was collected; ND, below the limit of detection of the method.
[a]Morning sampling on a day where two samples were obtained.
[b]Evening sampling on a day where two samples were obtained.
[c]Urinary measurements in parentheses are expressed in micrograms per liter.

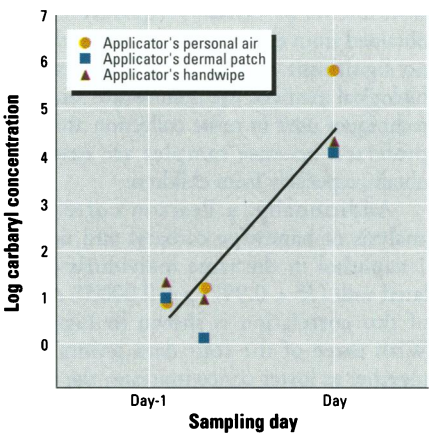

Figure 1. Graph of the log of environmental carbaryl measurements of an applicator before (Day -1) and the on the day (Day) of carbaryl application (R^2 = 0.85). The units of measurement are nanograms per cubic meter for personal air, nanograms per sample for dermal patches, and micrograms per 2 hands for handwipes.

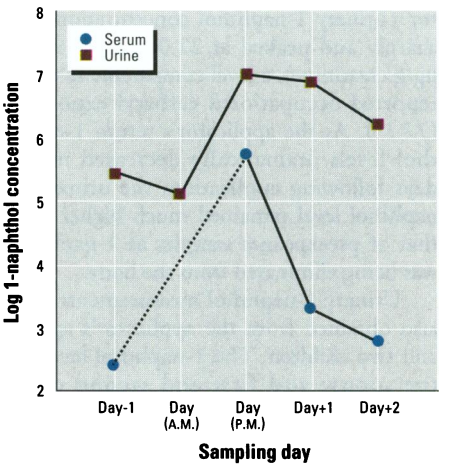

Figure 2. Carbaryl uptake and elimination in serum (measured as 1-naphthol) and urinary 1-naphthol excretion in a carbaryl applicator. All sampling days are shown relative to the application day (Day). Both morning and evening urine samples were obtained on the application day, whereas only an evening serum sample was obtained. The dashed line indicates that a data point (application day morning sample) is missing in the serum profile.

The results of the environmental measurements for the carbaryl applicator suggest overt occupational exposure (Fig. 1). All environmental measurements taken the day carbaryl was applied were significantly higher than those taken before application, and all environmental measurements appeared to increase at similar rates. A regression analysis of the sampling day versus the log of the carbaryl concentration of all environmental samples produced a coefficient of determination (R^2) of 0.85, indicating an agreement between the measurements. In addition, the urinary and serum 1-naphthol concentrations of biological samples obtained on the same days rise at similar rates as the environmental samples.

The biological measurements of samples from the carbaryl applicator showed a distinct elimination pattern (Fig. 2). The serum uptake/elimination profile was consistent with the expected profile (9). Before pesticide application, the serum 1-naphthol level of this applicator was higher than that of applicators from farms that did not apply carbaryl. Following carbaryl application, the serum 1-naphthol concentration of the applicator had increased by about three orders of magnitude, peaking at about 0.5 mg/l (ppm). In addition, approximately 100 ng/l nonmetabolized carbaryl

was detected in the application day serum sample from the applicator. His serum 1-naphthol level decreased in the 2 days following exposure until it closely approached the preapplication level.

The carbaryl applicator's urinary 1-naphthol level was consistently higher than his serum 1-naphthol level (Fig. 2). The creatinine-adjusted urinary 1-naphthol concentration in the applicator (preapplication sample) was about 250 µg/g creatinine, which is significantly greater than the 95% percentile reference range value of 36 µg/g creatinine (*10,11*). On the morning before application, the urinary 1-naphthol concentration dropped almost 50% from the previous day, although the level was still over three times the reference range. This decrease could possibly be a result of continued elimination from a previous exposure. After carbaryl application, the applicator's urinary 1-naphthol concentration rose sharply and peaked at 22,000 µg/l (9,300 µg/g creatinine), a level comparable to other reported occupational carbaryl exposures (*12,13*). As the applicator's serum 1-naphthol levels dramatically decreased in the days following application, the urinary 1-naphthol level remained much higher than that of preexposure samples as 1-naphthol was being eliminated from the body.

Urinary 1-naphthol measurements were also obtained from the applicator's spouse and two children. The 1-naphthol levels in the spouse and first and second child approximately doubled following carbaryl application (from 13, 7.4, and 8.1 µg/g to 27, 12, and 19 µg/g creatinine, respectively), indicating exposure to carbaryl during its application on their farm; however, the values were low and within the reference range of the U.S. population. Neither the indoor air concentration of carbaryl, which is representative of the personal air concentrations of the family members, nor the house dust levels appeared to increase upon application of carbaryl on the farm, so it is unlikely that this would be the source of the low-level exposure in family members. Even at the low levels observed, the exposure of family members to carbaryl applied on the farm warrants further study.

A Pearson correlation analysis of the log serum and log urinary 1-naphthol concentrations in the same individuals showed good correlation between the two methods (R^2 = 0.945, p = 0.0003). A plot of the method correlation is shown in Figure 3. The number of data points for this analysis is small; therefore, corresponding data may not be accurately interpolated from the resultant regression line. However, the good correlation between serum and urinary concentrations of 1-naphthol suggests that accurate

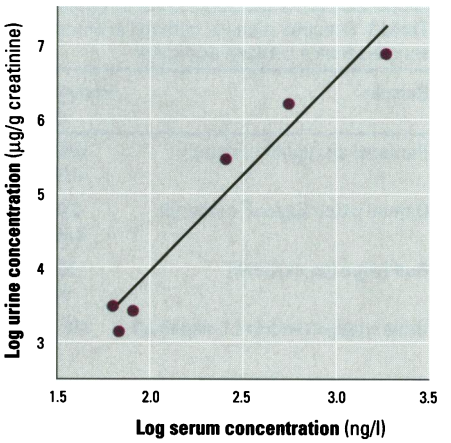

Figure 3. Correlation plot of serum and urinary 1-naphthol measurements. The correlation coefficient is 0.945 (n = 7, p = 0.0003).

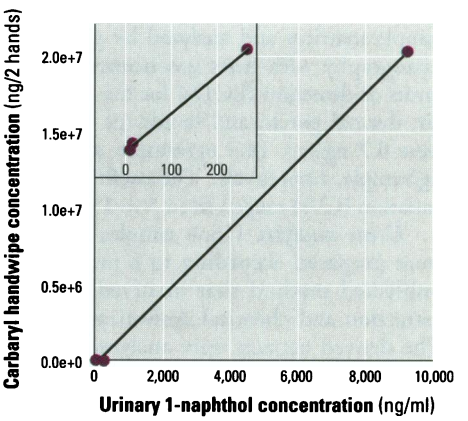

Figure 4. Correlation plot of handwipe carbaryl and urinary 1-naphthol measurements. The correlation coefficient is 0.997 (n = 4, p = 0.0003). The lower concentration end of the plot is enlarged in the inset.

assessments of carbaryl exposure can be obtained from either medium. When there is no significant measurement bias between biological matrices, the noninvasive sampling techniques used in urine collection are often preferred because samples are easier to obtain, especially from children.

Additionally, a Pearson correlation analysis of handwipe carbaryl and urinary 1-naphthol in the same individuals correlated well (R^2 = 0.997, p = 0.0003). A plot of this correlation is shown in Figure 4. With three of the four data points close together at lower concentrations, the correlation was largely determined by a single data point. However, as shown in the inset (Fig. 4), the data correlate well (R^2 = 0.996, p = 0.003) even when the high value is omitted. This correlation suggests that handwipe measurements may accurately reflect the change in internal dose of carbaryl following an exposure.

Although primarily associated with carbaryl elimination, urinary 1-naphthol is also attributed to naphthalene exposure, especially from cigarette smoke (*14,15*). When 1-naphthol is mainly derived from naphthalene, a correlation usually exists between it and 2-naphthol, a secondary metabolite of naphthalene (*11*). A poor correlation between the two geometric isomers of naphthol is indicative of another 1-naphthol source, usually carbaryl exposure.

For statistical purposes, we sorted samples into two groups: an application and nonapplication group. Samples were in the application group if they were obtained from any person on the farm where carbaryl had been applied during the sampling week. Conversely, samples were in the nonapplication group if they were obtained from farms where carbaryl was not applied. Plots of log 1-naphthol concentration versus log 2-naphthol concentration in application and nonapplication samples are shown in Figure 5. A Pearson correlation analysis of each subgroup was performed (Fig. 5). The concentrations of urinary naphthol isomers among samples of the nonapplication group were significantly correlated (p = 0.0001), whereas the concentrations among samples of the application group were not (p>0.05). This supports the assumption that the increase in excretion of 1-naphthol in the urine of residents of a farm where carbaryl was applied is due mainly to the bodily absorption of carbaryl during the application period.

Conclusion

In most cases, the most useful exposure information is the biologically effective dose of a toxicant; however, this information is usually difficult, if not impossible, to obtain. In contrast, environmental measurements are usually easy to obtain and can be good indicators of the actual intake of a pesticide, especially if multiple intake routes are evaluated. However, the most direct indicator of the intake of toxicants during an exposure is the measurement of the body burden or internal dose of toxicants or their metabolites in blood, urine, or tissues. Because metabolic rates may vary from person to person, blood or serum measurements of the nonmetabolized toxicants are often considered more accurate than urinary metabolite measurements. However, urine samples are much easier to obtain and are often the only samples available from small children.

In this study, the usefulness of both environmental and biological data in evaluating environmental exposures has been demonstrated. The environmental data of the carbaryl applicator would have been

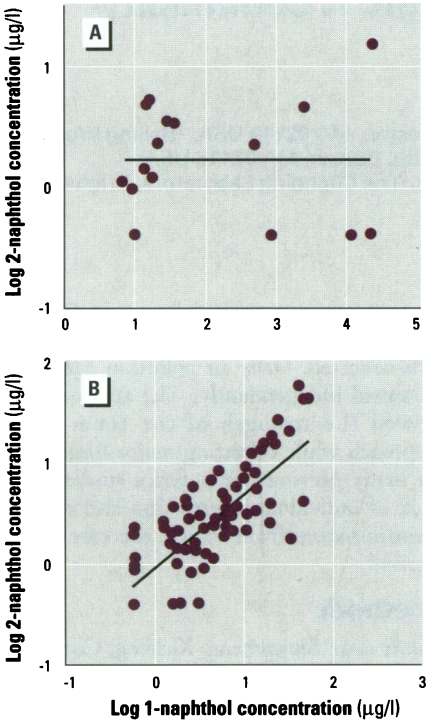

Figure 5. Correlation plots of naphthol isomers. The naphthol isomers are poorly correlated in the application group (A) (R^2 = 0.22, p>0.05), indicating a non-naphthalene source of urinary 1-naphthol. The naphthol isomers correlate well in the nonapplication group (B) (R^2 = 0.62, p = 0.0001), indicating primarily naphthalene-derived 1-naphthol.

sufficient to decide whether an exposure had occurred. However, biological measurements were necessary to determine the extent of the toxicant absorbed in each member of the farmer applicator's family. A strong correlation between serum and urinary 1-naphthol measurements has been shown. This correlation indicates that either matrix can be used to accurately evaluate occupational exposure. In theory, since there was good agreement among the environmental and biological measurements, additional data points could be used to construct a curve from which environmental measurements could be used to estimate body burden.

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